

(FILE 'HOME' ENTERED AT 12:09:47 ON 15 FEB 2005)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 12:09:50 ON 15 FEB 2005

L1 9971 S BACILLUS (3N) MEGATERIUM
L2 1057 S BM-3 OR BM3
L3 906 S (L1 OR L2) AND (P-450 OR P450)
L4 245 S L3 AND (MONOOXYGENASE OR MONO-OXYGENASE)
L5 62 S L4 AND (VARIANT OR MUTANT OR MODIFI?)
L6 49 S L5 AND (HYDROXY?)
L7 29 DUP REM L6 (20 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 12:18:44 ON 15 FEB 2005

L8 0 S L5 AND (87 OR 47 OR 188 OR 26 OR 72 OR 354)

FILE 'MEDLINE, CAPLUS, BIOSIS, AGRICOLA' ENTERED AT 12:26:43 ON 15 FEB 2005

L9 4 S L5 AND (87 OR 47 OR 188 OR 26 OR 72 OR 354)
L10 2 DUP REM L9 (2 DUPLICATES REMOVED)

=>

	Type	Hits	Search Text
1	BRS	1	"20030119046"
2	BRS	2065	monooxygenase
3	BRS	11	S65 near10 megaterium
4	BRS	4	S65 near10 (bm-3 or bm3)
5	BRS	2195	(bacillus near2 megaterium) or (bm-3) or bm3
6	BRS	71	S68 and monooxygenase
7	BRS	51	S69 and "87"
8	BRS	37	S68 near10 P450
9	BRS	4	"6100074"
10	BRS	29	FLITSCH and wong
11	BRS	349	(mono-oxygenase or monooxygenase) and cytochrome and P-450
12	BRS	991	(mono-oxygenase or monooxygenase) and cytochrome and (P-450 or P450)
13	BRS	130	S75 and (megaterium or bm3 or bm-3 or bm)
14	BRS	12	p450bm-3
15	BRS	2140	wong and bell
16	BRS	10	wong and bell and carmichael
17	BRS	0	"3825421.2"
18	BRS	10	"3825421"
19	BRS	67	wong and bell and p450
20	BRS	5	wong and p450bm-3

L7 ANSWER 12 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
 AN 2000:368602 CAPLUS
 DN 133:14090
 TI Enzymic **hydroxylation** of terpenes using substituted cytochromes
P 450 in fusion proteins with electron donor systems
 IN Wong, Luet Lok; Bell, Stephen Graham; Carmichael, Angus Bishop
 PA Isis Innovation Ltd., UK
 SO PCT Int. Appl., 63 pp.
 CODEN: PIXXD2
 DT Patent
 LA English
 FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 2000031273	A2	20000602	WO 1999-GB3873	19991119
	WO 2000031273	A3	20000817		
	W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
	RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
	EP 1131440	A2	20010912	EP 1999-956167	19991119
	R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO			
	JP 2002530112	T2	20020917	JP 2000-584082	19991119
PRAI	GB 1998-25421	A	19981119		
	WO 1999-GB3873	W	19991119		

AB A process for oxidizing a substrate which is an acyclic or cyclic terpene, or a cycloalkene; or a substituted derivative thereof, which process comprises oxidizing said compound with a **mutant** heme-containing enzyme, the **mutant** comprising the substitution of an amino acid in the active site by an amino acid with a less polar side-chain. The enzyme is typically P 450cam, the camphor **monooxygenase** cytochrome P 450 of Pseudomonas putida or P 450BM-3 from **Bacillus megaterium**. Cells and libraries of cells in which the process can be carried out or which can be used to select advantageous **mutant** enzymes are also provided. The method uses a cytochrome P 450 fusion protein with its electron donor system, e.g. the P 450 of Pseudomonas putida in combination with putaredoxin and putaredoxin reductase, manufactured using a high level expression construct in Escherichia coli.

L7 ANSWER 19 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
 AN 1997:709125 CAPLUS
 DN 128:58875
 TI Engineering the substrate specificity of *Bacillus megaterium* cytochrome P-450 BM3: **hydroxylation** of alkyl trimethylammonium compounds
 AU Oliver, Catherine F.; Modi, Sandeep; Primrose, William U.; Lian, Lu-Yun; Roberts, Gordon C. K.
 CS Cent. Mech. Human Toxicity, Leicester, LE1 9HN, UK
 SO Biochemical Journal (1997), 327(2), 537-544
 CODEN: BIJOAK; ISSN: 0264-6021
 PB Portland Press
 DT Journal
 LA English
 AB Oligonucleotide-directed mutagenesis was used to replace Arg-47 with Glu in cytochrome P 450 BM3 from *B. megaterium* and in its heme domain. The **mutant** protein was characterized by sequencing, mass spectrometry, steady-state kinetics, and by optical and NMR measurements of substrate binding. The **mutant** protein retained significant catalytic activity toward C12-C14 fatty acids, catalyzing **hydroxylation** in the same (ω -1, ω -2, ω -3) positions with k_{cat}/K_m values a factor of 14-21 lower. C12-C16 alkyl trimethylammonium compds. were relatively poor substrates for the wild-type enzyme, but were efficiently **hydroxylated** by the R47E **mutant** at the ω -1, ω -2, and ω -3 positions, with k_{cat} values of up to 19 s⁻¹. Optical spectroscopy showed that the binding of the C14 and C16 alkyl trimethylammonium compds. to the **mutant** was similar to that of the corresponding fatty acids to the wild-type enzyme. Paramagnetic relaxation measurements showed that laurate bound to the ferric state of the **mutant** in a significantly different position, 1.5 Å closer to the Fe atom, than seen in the wild-type protein, although this difference was much smaller (.apprx.0.2 Å) in the ferrous state of the complex. The binding of a substrate having the same charge as residue 47 to the ferric state of the protein was roughly 10-fold weaker than that of a substrate having the opposite charge (and thus was able to make an ion-pair interaction with this residue). The results were discussed in the light of the 3-dimensional structure of the protein.

AN 93216707 MEDLINE
DN PubMed ID: 8463285
TI Critical residues involved in FMN binding and catalytic activity in cytochrome P450BM-3.
AU Klein M L; Fulco A J
CS Department of Biological Chemistry, School of Medicine, University of California, Los Angeles 90024-1737.
NC GM23913 (NIGMS)
HL-07386 (NHLBI)
SO Journal of biological chemistry, (1993 Apr 5) 268 (10) 7553-61.
Journal code: 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199305
ED Entered STN: 19930521
Last Updated on STN: 19930521
Entered Medline: 19930505
AB Cytochrome P450BM-3 from *Bacillus megaterium* is a soluble, catalytically self-sufficient fatty acid **mono-oxygenase** that, in structural organization and amino acid sequence, resembles the Class II (microsomal) **P450** systems. Its single polypeptide chain contains both a **P450** heme domain and an **NADPH:P450** reductase domain, each of which bears significant homology with its microsomal counterparts. We report here the critical nature of three amino acids in the reductase domain of this enzyme with respect to FMN binding and catalytic activity. We used site-directed mutagenesis to change glycine 570 to bulkier amino acids; none of these **mutant** enzymes contained FMN after purification. We also made substitutions for tryptophan 574 and tyrosine 536, which by sequence analogy (Porter, T. D. (1991) Trends Biochem. Sci. 16, 154-158) were proposed to bind FMN through stacking of the aromatic rings with the isoalloxazine ring of the flavin. **Mutants** of tryptophan 574 which retained the aromatic side chain contained no less than 0.85 mol of FMN per mol of enzyme, while aspartate and glycine substitutions yielded enzymes which did not incorporate FMN. Substitution of tyrosine 536 with aspartate gave an enzyme which contained 0.44 mol of FMN per mol of enzyme but was inactive as a fatty acid **hydroxylase** and had only 2% of wild-type cytochrome c reductase activity, while the glycine **mutant** at this position bound no FMN. Furthermore, although all of the **mutant** enzymes contained 1 mol of FAD per mol of enzyme, the Y536D **mutant** and those entirely lacking FMN retained no more than 40% of wild-type ferricyanide reductase activity. By assaying these enzymes in the presence of added FMN, we were able to assess the relative importance of the residues in the wild-type sequence with respect to their contribution to FMN binding. In addition, the aromatic **mutants** of tryptophan 574, which were nearly as active in cytochrome c reduction as wild-type P450BM-3, were only 20% as active in myristate **hydroxylation** as the wild-type enzyme, suggesting that this amino acid plays an important role in the flow of electrons between the **P450** heme and reductase domains.

L7 ANSWER 27 OF 29 CAPLUS COPYRIGHT 2005 ACS on STN
 AN 1994:72136 CAPLUS
 DN 120:72136
 TI Cytochrome P450BM-3 (CYP102): Regiospecificity of oxidation of
 ω -unsaturated fatty acids and mechanism-based inactivation
 AU Shirane, Noboru; Sui, Zhihua; Peterson, Julian A.; Ortiz de Montellano,
 Paul R.
 CS Dep. Pharm. Chem., Univ. California, San Francisco, CA, 94143-0446, USA
 SO Biochemistry (1993), 32(49), 13732-41
 CODEN: BICHAW; ISSN: 0006-2960
 DT Journal
 LA English
 AB Cytochrome P 450BM-3 [fatty acid ω -(1-3)- **monooxygenase**]
 (I) of **Bacillus megaterium** preferentially oxidized
 fatty acids with terminal double or triple bonds to the ω -2-
hydroxylated fatty acids rather than resp. to the epoxide or
 diacid metabolites. I was inactivated during catalytic turnover of long,
 terminally unsatd. fatty acids but not by the analogous medium-length
 fatty acids. I inactivation by 17-octadecynoic acid and 16-
hydroxy-17-octadecynoic acid was due to alkylation of the
 prosthetic heme group to give an adduct tentatively identified as
 N-(2-oxo-3-**hydroxy**-17-carboxyheptadecyl)protoporphyrin IX by its
 chromatog. and spectroscopic properties. Catalytic turnover of
 17-octadecenoic acid also resulted in heme **modification**. Fatty
 diacid monoethyl thioesters were introduced as a new class of irreversible
 inhibitors that exploit the ω -2 oxidation specificity of I. Catalytic
 oxidation of the monoethyl thioesters of dodecanedioic and hexadecanedioic
 acids resulted in I inactivation and formation of the parent diacids as
 metabolites. Limited tryptic digestion of I after incubation with the
 monoethyl thioester of [14C]hexadecanoic acid showed that the inactivating
 agent bound covalently to both the heme and flavin domains. This finding,
 and the observation that glutathione prevents inactivation of the enzyme
 by the monoethyl thioesters, indicated that a diffusible metabolite,
 probably the sulfoxide, was responsible for I inactivation. The strong
 preference for ω -2 allylic or propargylic **hydroxylation**
 over terminal π -bond oxidation was opposite to the usual cytochrome
 P 450 pattern and required that the enzyme actively
 suppress terminal π -bond oxidation The inference that I binds and
 sequesters the terminal C atom in a lipophilic pocket was consistent with
 the crystal structure of the hemoprotein domain of I.

L7 ANSWER 23 OF 29 MEDLINE on STN DUPLICATE 7
 AN 95399410 MEDLINE
 DN PubMed ID: 7669780
 TI Role of the linker region connecting the reductase and heme domains in cytochrome P450BM-3.
 CM Erratum in: Biochemistry 1996 Jul 2;35(26):8794
 AU Govindaraj S; Poulos T L
 CS Department of Molecular Biology and Biochemistry, University of California, Irvine 92717, USA.
 SO Biochemistry, (1995 Sep 5) 34 (35) 11221-6.
 Journal code: 0370623. ISSN: 0006-2960.
 CY United States
 DT Journal; Article; (JOURNAL ARTICLE)
 LA English
 FS Priority Journals
 EM 199510
 ED Entered STN: 19951026
 Last Updated on STN: 19980206
 Entered Medline: 19951013
 AB Cytochrome P450BM-3 is a fatty acid **monooxygenase** that contains the catalytic **P450** heme domain covalently attached to a diflavin **P450** reductase domain. The function of the linker region connecting the C-terminal end of the heme domain to the N-terminal end of the reductase domain has been studied by deleting parts of the linker and changing the sequence of the linker. Deleting three or six residues or changing an Arg-Lys-Lys stretch in the middle of the linker to Ala-Ala-Ala does not alter the functional properties of either domain. The **mutants** retain full cytochrome c and ferricyanide reductase activities characteristic of the **P450** reductase domain. The heme domain in the **mutants** retains its ability to bind a fatty acid substrate giving the full low-to-high spin shift and exhibits the normal 450 nm absorption band characteristic of the reduced carbon monoxide complex. However, the six amino acid deletion **mutant** exhibit nearly undetectable levels of fatty acid **hydroxylase** activity, the three amino acid deletion **mutant** about 10% activity, and the three Ala substitution **mutant** about 50% activity. The **mutants** also exhibit slower rates of reductase-to-heme electron transfer rates that correlate with the loss in fatty acid **hydroxylase** activity. These results indicate that the length of the linker and, to a much less extent, the sequence are important for correctly orienting the reductase and heme domains, which apparently is necessary to achieve efficient reductase-to-heme electron transfer rates.